Recapitulation of foregoing Experiment.

10,675 lbs. leady bismuth, holding five per cent. lead, yielded 9306 lbs. of good commercial bismuth by the crystallization process, or within six per cent. of the total contents of pure bismuth.

Leaving for subsequent treatment—

Of alloy, holding 40 per cent. of lead, 1188 lbs., which is equal to 11·13 per cent. of the whole weight of metal treated.

Average Analysis of the Bismuth Ores worked upon.

Bismuth	44·57
Lead	2·35
Antimony	0·64
Arsenic	1·26
Molybdenum	5·02
Tellurium	0·17
Iron	5·25
Manganese	0·05
Copper	0·24
Tungstic acid	2·45
Alumina	0·18
Magnesia	0·09
Lime	0·81
Carbonic acid	1·47
Sulphur	3·77
Insoluble earthy matter, chiefly silica	23·12
Water	3·37
Oxygen in combination and loss	5·19
	100·00

II. "An Inquiry into the Cause and Extent of a special Colour-Relation between certain exposed Lepidopterous Pupæ and the Surfaces which immediately surround them." By EDWARD B. POULTON, M.A., of Jesus and Keble Colleges, Oxford, Lecturer in Zoology and Comparative Anatomy at St. Mary's Hospital, Paddington. Communicated by Professor E. RAY LANKESTER, F.R.S. Received February 10, 1887.

(Abstract.)

Historical.—Mr. T. W. Wood first called attention to the colour-relation in pupæ ('Entom. Soc. Proc.,' 1867, p. xcix), adducing

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instances of *Pieris brassicæ*, *P. rapæ*, *Vanessa polychloros*, and (erroneously) *Papilio machaon*. He even suggested that gilded surfaces might probably be found to produce gilded pupæ, but the experiment has never been made until the present investigation. His observations were disputed by many entomologists, but were confirmed by Mr. A. G. Butler and Professor Meldola ('Zool. Soc. Proc.,' 1873). Finally, Mrs. Barber ('Entom. Soc. Trans.,' 1874, p. 519) obtained striking results with the pupæ of *Papilio nireus* (South Africa) which were confirmed by Mr. Roland Trimen, who experimented upon *Papilio demoleus*. Still later Fritz Müller ('Kosmos,' vol. 12, p. 448) argues that the dimorphic pupæ of *Papilio polydamus* do not possess the colour-relation. It was generally assumed that all the above instances of the colour-relation were to be explained by supposing the skin of the freshly formed pupa to be "photographically sensitive," but the explanation was never tested by any system of transference to other colours, and Professor Meldola pointed out in 1874 that there was no real analogy with photography. Furthermore, the explanation failed to account for the colour of pupæ which threw off the larval skin on a dark night. I therefore thought that the problem would probably prove to be essentially physiological, and that the reflected light would be found to act on the larva at some time before pupation and not upon the pupa itself, and it seemed probable that the sensitive area might be defined by experiment. The investigation was conducted in the summer and autumn of 1886.

I. *Experiments upon Vanessa Io.*—Material was kindly supplied by Mr. E. D. Y. Pode, of Slade, Ivybridge. Six mature larvæ were placed in a glass cylinder surrounded by yellowish-green tissue-paper, and all suspended themselves from the paper roof. Five changed into the rarer yellowish-green form of pupa, and the sixth immediately after the skin had been thrown off and while still moist and with the shape unformed, was transferred to a black surface in darkness, but the pupal colours deepened into a yellowish-green tint exactly like that of the other five pupæ.

This experiment, so far as it went, confirmed my anticipation of larval as opposed to pupal susceptibility, and added another striking instance of pupal colour-relation. Mr. W. H. Harwood, of Colchester, also informed me that he had found the same variety of this species on the under side of nettle leaves, but not the dark form which occurs commonly on walls, stones, &c. Hence the protective value of the colour-relation is well seen; the species having varieties suitable for vegetal and mineral surroundings, and adjustable by the stimulus supplied by the colours of the environment.

II. *Experiments upon Vanessa urticæ.*—This species was investigated in great detail, over 700 individuals being employed in the experiments. Material was in part supplied by Mr. Pode, but chiefly

found near Oxford. The first necessity was the construction of a standard list of colours with which to compare the pupæ which had been the subjects of experiment. The pupæ are very variable, and in many of the experiments the colour influence was only allowed to act during a small part of the time during which the larvæ are sensitive. Hence the careful record of minute differences was absolutely necessary, and the standard list was made as detailed as possible. The list was as follows:—

The degree of colour represented by—

(1.) Very dark, from the large amount of cuticular pigment; no gilding or the merest trace.

(2.) Dark normal form, but not so black as (1) and sometimes more gilding but very little.

(3.) Light normal form, sometimes with a fair amount of gilding, often with a predominant pinkish tint. This degree was afterwards subdivided into dark (3), (3), and light (3), and even further in certain experiments.

(4.) Very light variety, often extremely golden; sometimes light pink.

(5.) The lightest variety; often completely covered with the gilded appearance.

In the experiments summarised below, the individuals belonging to different companies were always separated, except in the larvæ subjected to green surroundings, so that the errors from varying hereditary tendencies were reduced to a minimum, for the larvæ of each company are hatched from the eggs laid by a single butterfly.

1. *The Results of Different Colours.*—*Orange* surroundings produced no effect, as far as the experiment went, for the few pupæ were all (3) and therefore showed no relation to the colour of the environment. After the experiment upon the allied *V. Io* I tried the effects of *green* upon a large number of individuals, but the resulting pupæ were on the whole rather darker than usual, probably because of the amount of shade produced by the tissue-paper. This conclusion suggested the use of *black* surroundings, and at once an immense effect was witnessed. These effects in turn suggested the use of *white* surroundings (white paper and white opal glass) and here also a powerful influence was exerted, the pupæ being often brilliantly golden in appearance. But it was clear that the very dark varieties were much better protected against the black surfaces than the lustrous golden pupæ against the white surfaces, and this consideration suggested the use of a material with which the golden appearance harmonised most perfectly, *i.e.*, metallic gold. Boxes and cylinders lined with gilt paper and turned towards a strong light produced the most extremely gilded varieties in a large proportion of the pupæ, and the metallic appearance was yellower and more truly golden than in the more silvery forms

produced by the use of white surroundings. The totals obtained by the use of these different surroundings were as follows (omitting the orange).

Degrees of colour.	(1.)	(2.)	Dark (3.)	(3.)	Light (3.)	(4.)	(5.)	
Green surroundings	2	8	..	25	..	1	3	= 39
Black ,, ...	11	29	27	22	14	2	..	=105
White ,, ...	..	7	21	37	44	25	11	=145
Gilt ,, ...	..	1	2	7	16	27	14	= 67
								356

2. *The Colours of Wild Pupæ.*—It is impossible to realise the extremely remarkable results of the gilt and white surroundings without taking into account the fact that a (4) or a (5) is very rarely seen in the field, except when the pupa is diseased. Out of fifteen wild pupæ found August 31 on a grey stone wall, the lightest pupæ were (3), while there were four of the degree represented by (1), and there was only the minutest spot of gold to be seen after careful examination on two of the pupæ, and none on any of the others.

3. *The Effects of Mutual Proximity.*—Inasmuch as the above figures show that the larvæ are sensitive to dark surfaces and the larvæ themselves are almost black, it appeared probable that they would be mutually influenced when a large number pupated in close proximity. This was incidentally shown to be the case in several of the experiments, of which the most striking was as follows. Four larvæ were placed each in a separate cylinder while twelve were placed together in another similar cylinder, all having the same conditions of light and each cylinder lined and roofed with an equal amount of white paper and each standing upon an opal glass floor. Of the twelve larvæ ten pupated in close proximity upon the roof and sides, and were all light (3), while the remaining two pupated on the floor and were both (4). Of the four pupæ in separate cylinders two were (4) and two were (5). In consequence of these and other equally convincing results the exact position of the pupæ has to be taken into account in estimating the influences which have been at work, and in all the most careful experiments only one or two larvæ were placed in each coloured case.

4. *The Effects of Illumination.*—One experiment was directed towards the comparison of the influence of a black surface in strong light and the same surface in darkness, and the results show clearly

that the pupæ are on the whole darker in the latter circumstances, although dark under both conditions.

In another experiment some larvæ were suspended in a strong direct light without any coloured background sufficiently near to affect them, and as far as the experiment went it indicated that there was an influence in the direction of the lighter varieties, but in this case the numbers employed were too small to be convincing.

5. *The Time during which the Larvæ are Sensitive.*—The whole period preparatory to pupation, intervening between the cessation of feeding and pupation itself, may be divided into three stages: (i) in which the larva descends from the food-plant and wanders about in search of some (generally mineral) surface upon which to pupate; (ii) in which it rests motionless, usually in a curved position, upon the surface selected; (iii) in which it hangs head downwards suspended by its posterior claspers from a boss of silk spun at the close of the last stage. The duration of Stage (i) depends upon the varying proximity of suitable surfaces, and it was always greatly curtailed in confinement, because such surfaces were close at hand. If the larva is sensitive during this stage, the influences cannot generally contribute towards the result, because the larva is wandering over surfaces of various colours. It is also very improbable that the larva can be sensitive after the first few hours, or at any rate the first half of Stage (iii), because rapid changes are taking place under the larval skin, and it is even likely that processes are already on their way towards completion which will result in the formation of pigment or other substances, which will many hours later deepen into the effective causes of pupal colour. The length of Stage (iii) did not vary very much in different larvæ, and in the shortest case observed the length was about 14 hours, while 20 hours was an unusually long period, but the majority of larvæ passed about 17 or 18 hours in this stage. Stage (ii) was more variable, but about 15 hours was a common length, while 36 hours is a fair estimate of the length of the whole preparatory period. In the majority of cases a larva is probably sensitive to the colour of surrounding surfaces for about 20 hours preceding the last 12 hours of the whole preparatory period. Thus the length is amply sufficient to include many hours of daylight during which the surrounding surfaces are illuminated. If a larva be disturbed when Stage (ii) is far advanced the whole period begins again, and all the three stages are again passed through, but they are all abbreviated, including Stage (iii), which had not previously commenced. Many experiments indicated that darkness may increase the length of the stages; but my observations were not specially directed towards the settlement of this question, which only occurred to me when the notes were tabulated. Therefore I propose to specially investigate this point in the next season. Such prolongation,

if corroborated, may be physiologically connected with pigment formation, or it may merely give the larva an additional opportunity of being acted on by light, if for any cause the illumination of the surrounding surface is delayed, or if the most sensitive part of the whole period corresponds to the ordinary darkness of night.

6. *Experiments which show the Sensitive Condition during Stages* (ii) *and* (iii).—It was very important to obtain beyond any doubt the demonstration that the larvæ are sensitive during Stage (ii), and also to decide conclusively whether any susceptibility was continued into Stage (iii), and if so to compare the relative susceptibilities of the two stages. Such experiments, if successful, would at once dispose of the older theory of pupal sensitiveness, and would be most important in making possible other methods of investigation which, applied to Stage (iii) alone, might successfully terminate the long and difficult search for the larval sensory surface which is affected by surrounding colours. A great many experiments were conducted with this object. The larvæ were made to pass Stages (i) and (ii) exposed to the influence of a powerfully acting colour, and then were transferred for Stage (iii) to the colour which tended most strongly in the opposite direction. The largest, most carefully conducted, and most successful experiment of the kind gave the following results, all the larvæ belonging to the same company:—

Degrees of colour.	(1.)	(2.)	Dark (3.)	(3.)	Light (3.)	(4.)	(5.)	
In black surroundings for the whole period	..	1	..	5	..	1	..	= 7
Transferred from black into gold for Stage (iii)........	..	..	..	1	5	3	..	= 9
Transferred from gold into black for Stage (iii)	..	..	..	..	6	9	..	=15
In gold surroundings for the whole period	..	..	..	..	5	7	8	=20
								51

The analysis speaks for itself. Stages (ii) and (iii) are both sensitive, but Stage (iii) is much less sensitive than the other. Thus, when the earlier part of the period was passed in gilt surroundings, the resemblance between the results and those produced by gilt surroundings acting during the whole period was much stronger than the resemblance between the latter and the results produced when the gilt acted during Stage (iii) only. It is observable that the larvæ as a whole evidently tended towards the lighter forms, so that the black

did not produce nearly such strong effects as the gilt surroundings. It is almost unnecessary to point how completely the old theory of pupal sensitiveness is broken down by the analysis. The experiment shows that the more elaborate methods alluded to above could be applied to Stage (iii) with at any rate a fair prospect of success.

7. *The Search for the Sensitive Larval Surface.* (α.) *The Ocelli.*—The most obvious suggestion pointed towards the larval ocelli (six in number on each side of the head) as the possible sense-organs which were acted on by surrounding colours, and formed the beginning of the physiological chain of which the end is seen in the colours of the pupa. In many different experiments the larvæ were divided into two sets, with precisely similar conditions of surrounding colours and illumination, the one set of larvæ being normal while the ocelli of the other larvæ were carefully covered with an opaque black varnish, which was renewed more than once if necessary (the larvæ being very much irritated by the process and flinging their heads about so as to remove some of the varnish). The material made use of was a quickly-drying, photographic varnish, rendered opaque by the addition of lamp-black. Experiments of this kind were conducted with green, white, and gilt surroundings, but the pupæ which were formed from the blinded larvæ could never be distinguished as a whole from the others, having been equally acted upon by surrounding colours. Even supposing the conditions of experiment had not been quite perfect, so that the ocelli were not wholly eliminated, we should expect some differences between the resulting pupæ, if these organs represent the efficient sensory surface. After repeated experiments with negative results, I subjected two sets of larvæ to the influence of black surroundings in darkness, thinking it possible (but highly improbable) that the process of blinding, or the varnish itself, might act as a stimulus to the ocelli, and so produce the light-coloured pupæ. Again it was possible that the blinding might assist the influence of black surroundings, although it could not prevent the action of bright colours. Of the resulting pupæ, the set produced from the blinded larvæ were rather lighter than the others, but there was little difference, and hence both suggestions were negatived, for the process obviously did not assist the influence of the surroundings, and the difference between the two sets was so slight as to offer no explanation of the brilliant pupæ produced from blinded larvæ by gilt or white surroundings, on the hypothesis that the process of blinding itself supplies a stimulus.

(β.) *The Complex Branching Spines.*—It seemed possible that these spines, of which there are seven on most of the segments, might contain some terminal organ which receives impression from coloured surfaces. When the spines are snipped off the bases bleed a little, so it is clear that a subcuticular core is contained within them. The

bristles were shorn from several mature larvæ, and they were placed under exactly the same conditions of light and surroundings (white or gilt being used in three different experiments) as about an equal number of normal larvæ, but the pupæ of the two sets were almost exactly similar.

(γ.) *The whole Skin Surface as Tested by Conflicting Colour Experiments.*—It has been shown in paragraph 6 that the larvæ are to some extent sensitive during Stage (iii), and I had long thought that this stage in which the larvæ are suspended motionless, and cannot be greatly affected by disturbance, might be investigated by the application of strongly conflicting colours to different parts of the larval surface. Black and gilt were obviously shown to be the best colours to select for the purpose, and the experiments were conducted in two ways. In the first the larvæ were induced to suspend themselves from sheets of clear glass, by placing them in wide shallow glass boxes, so that the ascent to the glass roof was easily accomplished. As soon as suspension had taken place each of the larvæ was covered with a compartmented cardboard tube, of which the septum was perforated by a hole just large enough to admit the larval body. The tube was fixed to the glass sheet with glue, and the upper chamber and upper surface of the septum were lined with one colour, *e.g.*, gold, while the lower chamber and lower surface of the septum were lined with the opposite colour, *e.g.*, black, which also covered the outside of the cylinder, in case the larva should stretch beyond its lower edge. The septum was placed at such a height in the tube that the larval head and rather less than half of the total skin surface (anterior) were contained in the lower chamber, while rather more than half of the skin surface (posterior) was contained in the upper chamber. It was thought that the upper chamber would be illuminated too strongly as compared with the lower, because its opening was directed upwards towards the light descending from the window, and therefore compensation was provided by fixing another perforated septum on the upper end of the cylinder, so that its opening was reduced to the same diameter as the perforation in the septum between the two chambers. The results show that I overcompensated for the difference in illumination, for I did not take into account the fact that the larva spins its boss on a comparatively wide layer of silk which it has previously spun over the glass, and which greatly diminishes the transparency of the latter over an area at least equal to the diameter of the tube, which is comparatively thick, and includes the boss itself over the smaller area, corresponding to the perforation in the disk. Hence the resulting pupæ were rather lighter when the gilt chamber was below, although the difference was not great. At the close of the experiment I altered the conditions of illumination by removing the upper septum, and then the single pupa

produced in a tube with the gilt chamber above proved to be the only (5) obtained in the whole of this set of experiments, in which 83 pupæ had been compared. Such results show that the sensitive surface is not represented by a sense-organ in the head, or with an anterior portion only, but that the whole skin area possesses susceptibility.

The second method of conducting conflicting colour experiments was superior in the more equal illumination of the gilt surface when above or below. Flat wooden trays were covered in each case with black and gilt paper in alternating areas, the two colours meeting along lines which ran across each tray, and along which shelves were fixed covered with gilt paper towards the gilt surface, and black paper towards the black surface. The shelves were perforated close to the tray bottom with holes separated by equal distances, and of such a size as to easily admit the body of a larva with its spines, while the latter as in the compartmented tubes tend to obscure the interval between the larval body and the edge of the aperture. The trays were placed vertically in a strong east light, so that the shelves projected horizontally, the black surface being uppermost in some cases, the gilt surface in others. Suspended larvæ were pinned (by the boss of silk) on to the uppermost colour in such a position over the holes that the head and first five segments of each larva passed through a hole into the colour beneath, which tended to produce opposite results. The curvature of the larval body brought the head close up to the underside of the shelf, and thus there was no chance of its being influenced by the colour above the shelf. Other larvæ were similarly fixed between the shelves upon one colour only, so as to afford a comparison with the results of the conflicting colours. The pupæ obtained were on the whole rather lighter when the gilt surface was above, and hence the gilt surroundings influenced the rather larger posterior part of the skin to a greater extent than in the converse arrangement, when the effective colour was below. Hence on the whole the influence of conflicting colours has ended in as complete a confirmation of the numerous blinding experiments as the necessarily limited conditions of experiment could be expected to produce.

8. *The Nature of the Effects Produced.*—The gilded appearance is one of the most metal-like appearances in any non-metallic substance. The optical explanation has never been understood. It has, however, been long known that it depends upon the cuticle, and needs the presence of moisture, and that it can be renewed, when the dry cuticle is moistened. Hence it can be preserved for any time in spirit. If a piece of dry cuticle be moistened on its upper surface the colour is not renewed, but almost instantly follows the application of spirit to the lower surface. Sections of the cuticle resemble those of *Papilio machaon* described in a previous paper ('Roy. Soc. Proc.,'

vol. 38, 1885, p. 279), and show an upper thin layer, and a lower much thicker, finely laminated layer, which is also striated vertically to the surface. With Professor Clifton's kind assistance I have been able to show that the appearances follow from interference of light, due to the presence of films of liquid between the lamellæ of the lower layer. The microscope shows brilliant red and green tints by reflected light, while in transmitted light the complementary colours are distinct, but without brilliancy. The latter colours are seen to change when pressure is applied to the surface of the cuticle, and when the process of drying is watched under the microscope, owing in both cases to the liquid films becoming thinner. In the dry cuticle the solid lamellæ probably come into contact, and prevent the admission of air, which, if present, would cause even greater brilliancy than liquid. The spectroscope shows broad interference bands in the transmitted light, which change their position on altering the angle of incidence of the light which passes through the cuticle. Precisely similar colours, metallic on reflection, non-metallic and with the complementary tints on transmission, with the same spectroscopic appearances and changes induced by the same means, are seen in the surface films which are formed on bottle glass after prolonged exposure to earth and moisture. In the alternating layers of the pupa the chitinous lamellæ are of higher, the liquid films of lower refractive index, hence water or alcohol produce brilliant appearances, while liquids of higher refractive indices produce less effect.

It is very interesting to note that this most specialised means of producing colour is probably derived in the most simple manner from the ordinary lamellated layer of other non-metallic pupæ (*e.g.*, *P. machaon*) in which the lamellæ merely act as reflectors, so that the pupa is brightly coloured by absorption due to pigment contained in the outer lamellæ only, and hence traversed twice by a large part of the incident light.

The dark pupæ of *V. urticæ*, and the dark parts of the brilliant pupæ, contain abundant pigment in the upper thinner layer only, which therefore acts as a screen, and shuts off light from the lamellated layer below, thus preventing the metallic appearance. In the brilliant pupæ this layer is transparent, and of a bright yellow colour, and doubtless assists in producing the yellowness of the golden appearance by absorption of light. The two layers are of different chemical constitution, for the upper will not stain in logwood, while the lower does so without difficulty.

9. *The Biological Value of the Gilded Appearance.*—Mr. T. W. Wood suggested that the appearance was so essentially unlike anything usually found in the organic kingdoms as to protect the organisms possessing it. Others have thought that it has the value of a warning colour, indicating an unpleasant taste. It is probable that it is now

used for this purpose, but it is improbable that such was its original meaning, for the fact that the appearance can be called up by the appropriate surroundings shows that it belongs to the highest class of protective colours, as far removed as possible from conspicuous warning colours, the object of which is to become as *unlike* their surroundings as possible. The former suggestion no doubt contains the true origin of the character, if we add to it the statement that the appearance is not only unlike anything organic, but strongly resembles many common mineral substances, especially the widespread mineral mica. The darker pupæ, on the other hand, resemble grey and weathered rock surfaces, just as the brilliant varieties resemble many exposed and recently fractured rocks. The shape of the Vanessa pupa is eminently angular and mineral looking. It is probable that the glittering form arose in a hot dry country, where exposed rocks would not weather for a long period of time. Gilded pupæ of Vanessa are formed from larvæ which contain parasitic larvæ of ichneumon flies, probably on account of the absence of pigment in such diseased individuals, and such absence being correlated with the gilded appearance, the latter is therefore formed. *Vanessa Io* has a green variety of pupa which appears when the insect is attached to its food-plant; *V. atalanta* has not such a form, and spins a tent of leaves when it pupates on the plant, while *V. urticæ* has neither the green variety nor the latter habit, and exhibits a strong disinclination to pupate among vegetal surroundings. During the past summer I only found three pupæ of the species on the food-plant in the field, and *all were* "*ichneumoned*" and were abnormally gilded.

III. *Experiments upon Vanessa atalanta.*—A few larvæ of this species, kindly sent me by Mr. J. L. Surrage, were subjected to gilt and to black surroundings, while a few others were left in bright light among the leaves of the food-plant. The results harmonised very completely with those obtained from *V. urticæ*, the first set of pupæ being uniformly golden, the second very dark and with hardly any or none of the gilded appearance, while the third were intermediate but nearer to the former. The length of Stage (iii) appeared to be about the same as in *V. urticæ*, as far as this could be ascertained from the limited data.

IV. *Experiments upon Papilio machaon.*—Mr. W. H. Harwood supplied me with larvæ of this species. The eight largest were selected and placed in brown surroundings (twigs, &c.), four of them being blinded. The larvæ were very quiet and did not appear to be irritated by the process, which was repeated three times. The position of the ocelli on a distinct black area rendered it easy to ensure that they are all covered with varnish. Eight bright green pupæ were obtained, fixed to the brown stems or roof or lying free on the brown floor. This result surprised me very much, for I knew that there

was a brown variety of the pupa not uncommon in this species. The remaining three larvæ were placed in green surroundings, one of them being blinded as above, but only one of the normal larvæ pupated, fixed to the green food-plant, and produced a *distinct brown variety*. These startling results show that there can be no susceptibility in this species, and this is all the more remarkable because the two varieties are so well marked, and because of the striking results obtained by Mrs. Barber and other observers on two species of South African Papilios. Fritz Müller, however, shows that another species of this genus resembles *P. machaon* in being dimorphic and yet not susceptible. The contradictory results obtained in my experiments were either due to the secondary association of one variety of a dimorphic species with an unhealthy condition or even a stunted size, as the gilded Vanessa pupæ result from "ichneumoned" larvæ, or to the shade caused by the green tissue-paper. The eight largest and healthiest larvæ produced the green pupæ, while of the three smaller larvæ only one pupated and formed a brown pupæ. Mr. Harwood informs me that he has always looked with suspicion on the brown pupæ, believing that they have been bred from larvæ which were captured when small, and which are reared in close-fitting tin boxes; and he believes that the wild pupæ, and those obtained from larvæ which were found when almost mature, are green. On the whole I think it is probable that the pupal dimorphism in this species is the remnant of a former suceptibility to coloured surroundings.

V. *Experiments upon Pieris brassicæ and P. rapæ.*—These two species are treated together because they were in nearly all cases kept under similar conditions and were often placed in the same cylinders. The (nearly mature) larvæ were almost always obtained, and the experiments conducted, at Seaview (Isle of Wight).

1. *Standards of Pupal Colour.*—Degrees of colour were constructed by the comparison of a large number of individuals in each species. In these standard lists the pupæ were arranged in both species according to the relative predominance of black pigment, both as patches and minute dots, the latter tending to produce a grey appearance and obscuring the ground colour. The lightest degrees were classified according to the tint of the ground colour which had become prominent in the comparative absence of the pigment.

2. *Effects of various Colours acting during the Preparatory Period.* (*α.*) *Black.*—Interesting results following the use of this black ground under various conditions of illumination (*P. rapæ* only), the effects being stronger in the direction of pigment formation when the amount of light was increased (the opposite effect having been witnessed in *V. urticæ*). The pupæ of both species were dark in the great majority of instances after exposure to black surroundings in the larval state during the preparatory period.

(β.) *White.*—In this case also the effects were stronger (as shown in the prevention of pigment formation) as the surface was more highly illuminated (*P. rapæ* only).

(γ.) *Colours of the Spectrum.*—All the colours were used except violet, and the effects upon pigment formation in the two species were so graduated in the successive colours that it was possible to approximately represent the results by a graphic method, making the abscissæ of the scale of wave-lengths of the visible spectrum, and each ordinate of a length which corresponded to the average amounts of pigment obtained from all the pupæ subjected to any one colour, each ordinate being made to diverge at its base, and to include the degrees on the scale of wave-lengths which were shown by the spectroscope to correspond to the rays reflected (or transmitted) by the colour in question. Joining the summits of all the ordinates, the lines obtained were strikingly similar in the two species.

The effects may be summarised as follows:—

Colours.	*P. brassicæ.*		*P. rapæ.*	
Black (for comparison).	Largest amount of pigment.		Largest amount of pigment.	
Dark red.	Almost the same.		..	
Deep orange.	Smallest amount of pigment.	Marked effects on tint of ground colour, especially in the case of *orange*.	Smallest amount of pigment.	Marked effects on tint of ground colour.
Pale yellow.	Rather more.		Rather more.	
Green.	Rather more.		Intermediate between the two last.	
Pale bluish-green.	Much more, almost equal to *black* and *red*.		More than in *yellow*.	
Dark blue.	.. ..		Still greater amount, but not nearly equal to *black*.	

The colours which most retard the formation of pigment were shown by the spectroscope to contain certain rays in common, *i.e.*, those from W.L. 0·00057—W.L. 0·00059, or 0·00060. The whole of the experiments on these species seemed to show that, of the light incident on the larval surface, the direct white light produces no effect at all (until after it has been reflected). Further experiments must decide whether direct light can be equally efficient with reflected light, when it contains the same spectroscopic components. The green tissue-paper was quite insufficient to prove this, for it must have been largely coloured by absorption from reflected as well as transmitted light.

3. *The Length of the Preparatory Period.*—The observations were not sufficient to determine the duration of the periods and of its stages with any great accuracy, but all the experiments render it certain that the length is much greater in both species than in *V. urticæ*. There were also some indications, as in *V. urticæ*, that darkness may cause the prolongation of the period.

4. *Blinding Experiments.*—The larvæ of *P. rapæ* were alone made use of, and they are as well suited to this method of investigation as *P. machaon*. The sets of pupæ produced from normal and blinded larvæ were very similar, and thus the results harmonise with those of all the blinding experiments in other species of larvæ.

5. *Transference Experiments.*—A considerable number of the larvæ of *P. rapæ* were transferred for the whole or part of Stage (iii) to a surface of a colour different from and generally opposite to that which had previously influenced them, and the results entirely harmonised with those previously described in other species, showing that the larva is sensitive and not the pupa, and that the time of greatest susceptibility is before Stage (iii), or only including the first part of it, but also rendering it probable that the larvæ can be influenced to a small extent during this stage.

6. *The Nature of the Effects wrought upon the Pupæ.*—The varied pigment effects which follow the influence of different surrounding colours are attended by other more deeply seated changes of even greater physiological interest and importance. The black pigment patches and minute black dots are cuticular and superficial, while the green, pink, or other ground colours are subcuticular and deep-seated, and in the most brightly coloured pupæ they are mixed colours, due to the existence of different pigmentary (and probably chlorophylloid) bodies present in different elements and at different depths in the subcuticular tissues of the same pupa. In other pupæ no trace of such colours can be seen. Hence we see in these most complex and varied effects of the stimulus provided by the reflected light, which deepen into their permanent pupal condition very many hours after the stimulus has ceased to act, the strongest evidence for the existence of a chain of physiological processes almost unparalleled in intricacy and difficulty, while a theory of comparatively simple and direct photochemical changes induced by the stimulus itself, without such a physiological circle, seems entirely inadequate as an explanation of the facts, a conclusion which is borne out by a comparison with the experiments upon other species described in this paper.

VI. *Experiments upon Ephyra pendularia.*—After the consideration of the many species of variable pupæ of the Rhopalocera, it is of interest to compare the results of the investigation of the equally exposed and variable pupæ of certain species of a single genus of Heterocera,

the genus Ephyra. I observed this genus in 1883 (*i.e.*, *E. pendularia*, *E. omicronaria*, and *E. orbicularia*), and the results are published in 'Entom. Soc. Trans.,' 1884, pp. 50—56. The most curious result of the observation was the establishment of the fact that the green and brown larvæ always produce pupæ of the same colour. I think it is very probable (from the consideration of other partially published observations), although entirely untested in this genus, that the colours of the larvæ, and through them of the pupæ, could be controlled by the selection of appropriate surroundings during the whole or a large part of the larval stage. Concerning the different species made use of, *E. orbicularia* is variable, *E. pendularia* regularly dimorphic green and brown, and *E. omicronaria* dimorphic, with the brown forms very rare. The relative numbers of the green and brown larvæ and pupæ of *E. pendularia* vary at different times of the year, the green forms greatly predominating in the summer brood, while they are not so abundant in the winter brood. When the parents of any set of larvæ were both of the same colour in the larval stage there was a much larger proportion of that same colour in the resulting offspring. I made some observations upon the situations selected for pupation, thinking that these might show some relation to the pupal colours, but the results were not convincing, and were certainly highly irregular, but the experiment was not carried out in the best way, for there was not a sufficient quantity of *both* colours in the surroundings. Dr. Wilhelm Müller, of Greifswald (Spengel, 'Zool. Jahrb.,' vol. 1, 1886, p. 234), calls attention to this remarkable and constant relation of larval to pupal colours, and expresses the belief that it is entirely exceptional, a statement which is of importance, when it is remembered that Dr. Müller has worked carefully for many years on the South American larvæ. Hence certain species of Ephyridæ afford an interesting contrast with all the other species of exposed pupæ which have been hitherto observed.

VII. *Experiments upon the Colours of the Cocoon in Saturnia carpini.*—At the suggestion of Mr. W. H. Harwood I made some experiments upon this species, and found that four cocoons which were spun in the corners of black calico bags were very dark brown in colour, while those of other larvæ which had been freely exposed to light until after they had begun to spin, and which were not surrounded by dark surfaces, were nearly all perfectly white, and when darker of a much paler tint, and very different from the four mentioned above. Thus Mr. Harwood's suggestion seems to be entirely confirmed, and another instance of the influence of surroundings is added, and one which it appears cannot be explained in any way except by the supposition of the existence of a complicated physiological, and apparently a nervous circuit.